Synthesis of Pyridine and Pyridinium Quinone Methide Precursors: Studies Towards the Realkylation of aged Acetylcholinesterase

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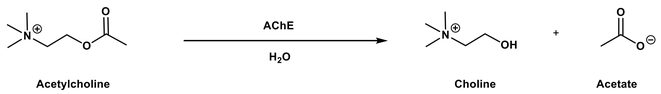
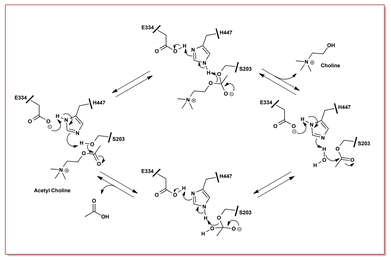
BACKGROUND

The treatment for exposure to chemical warfare agents, such as organophosphorus (OP) nerve agents that inhibit acetylcholinesterase (AChE) causing severe nerve damage, and in some cases death, is an important area of research.

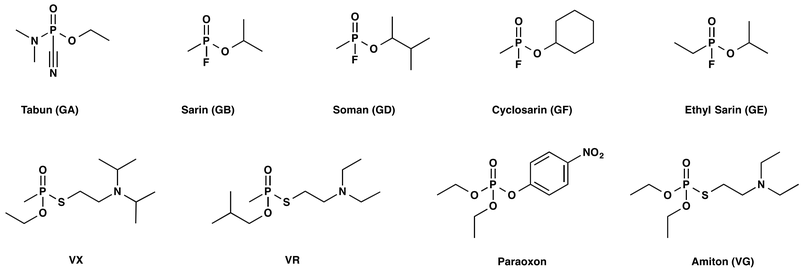


Each AChE hydrolyzes 25,000 molecules per second.
Active site residues: (Ser 203, His 447, Glu 334)

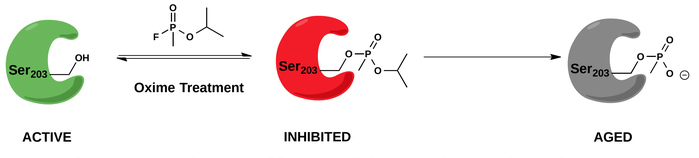
Inhibition
- Bradycardia
- Hypotension
- Hypersecretion
- GI tract hypermotility
- Decrease intraocular pressure
- Bronchoconstriction
- Prolonged muscular contraction



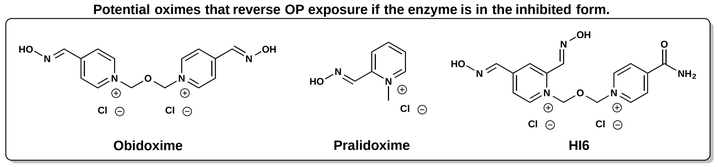
Organophosphorus (OP) Nerve Agents



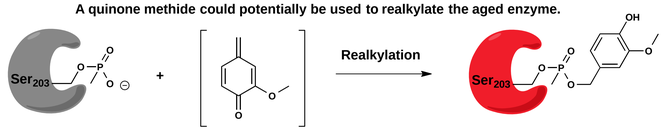
Organophosphorus Compounds Mode of Action



Potential oximes that reverse OP exposure if the enzyme is in the inhibited form.

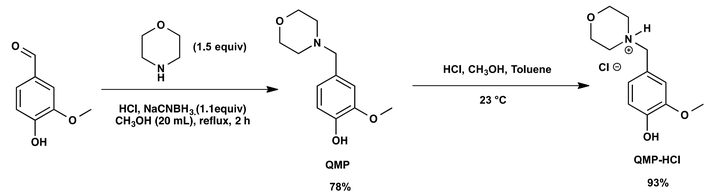


A quinone methide could potentially be used to realkylate the aged enzyme.



SYNTHESIS – FRAMEWORK 1

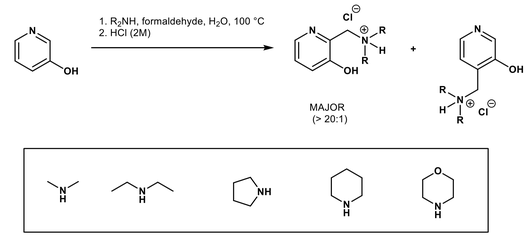
Goal: To successfully synthesize and characterize a library of *ortho/para*-quinone methide precursors (QMPs) derived from commercially available materials and to evaluate the nucleophilic reactivity of these precursors with various sulfur, oxygen and nitrogen-based nucleophiles.



Procedure: The reagents are combined in methanol and heated. During heating, the reaction begins with nucleophilic addition of the amine to the carbonyl of the aldehyde. The resulting intermediate is then reduced by the sodium cyanoborohydride to form the amine product. Protonation is then performed following extraction. The pronated QMP is finally isolated from any impurities using trituration.

SYNTHESIS – FRAMEWORK 2

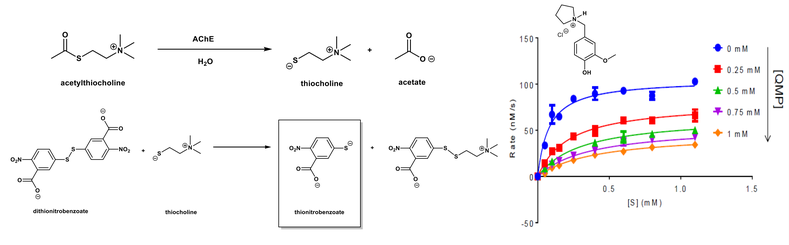
Goal: To expand our library of QMP's by synthesizing a number of pyridine based compounds. The computational methods that have been used to guide synthetic efforts have shown promise in these compounds.



Procedure: The reagents are combined in water and heated. During heating, the amine first participates in a Mannich reaction with the formaldehyde to form an electrophile. The pyridol ring then attacks the newly formed electrophile through an EAS pathway. The resulting QMP is extracted and protonated to give the QMP-HCl.

UV STUDIES – ELLMAN'S ASSAY

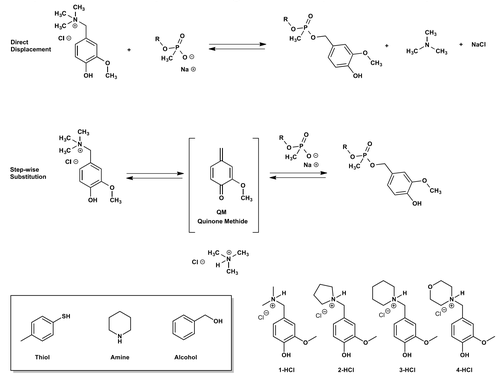
Goal: To determine if a QMP of interest is a competitive inhibitor via UV-vis monitoring of Ellman's assay.



Procedure: Ellman's assay uses the thiol ester acetylthiocholine (ATC) in place of acetylcholine. The ATC is hydrolyzed by the enzyme AChE to produce thiocholine and acetate. The thiocholine then reduces the dithionitrobenzoate (DTNB) to ultimately give thionitrobenzoate (TNB) which absorbs at 405 nm. When the QMP of interest in added into the assay, the change in absorbance can be measured via UV-vis in order to determine if the QMP is in fact a competitive inhibitor.

SCREENING PROCESS

Goal: To screen our library of protonated quinone methide precursors prior to testing in the AChE enzyme active site by evaluating the nucleophilic reactivity of each with various nucleophiles.

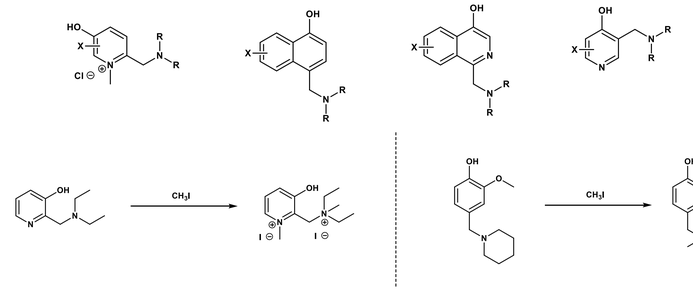


QMP	Nucleophile	% Yield
1-HCl	Thiol	83
	Amine	78
	Alcohol	8
2-HCl	Thiol	83
	Amine	78
	Alcohol	12
3-HCl	Thiol	85
	Amine	81
	Alcohol	10
4-HCl	Thiol	84
	Amine	69
	Alcohol	16

Procedure: A small amount of protonated QMP is combined in solution with a nucleophile and heated. After the heating period, the reaction is diluted and analyzed via GC/MS. Though the protonated starting material is not visible using this instrument, it allows us to see the neutral products and quantify the conversion using an internal standard.

FUTURE WORK

To continue to expand our library of quinone methide precursors by varying substituents on the aromatic ring and amine leaving group, and by synthesizing new framework compounds from the templates shown below



We would also like to methylate a number of our current frameworks and investigate their activity with the enzyme along with the proposed new frameworks.

ACKNOWLEDGEMENTS AND REFERENCES

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